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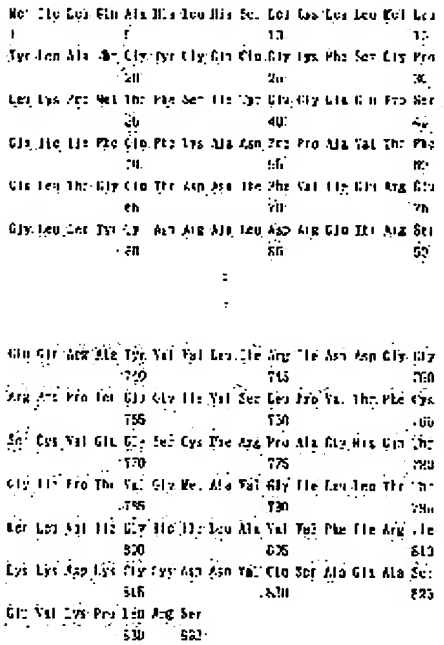
(54) MAMMALIAN INFLUX PEPTIDE TRANSPORTER

(57)Abstract:
PURPOSE: To obtain a new DNA useful for expression of mammalian influx peptide transporter activities for measuring oral bioavailability of a medicine.
CONSTITUTION: This DNA compound is an isolated one containing a DNA sequence encoding human influx peptide transporter activities, and, for example, containing the DNA sequence encoding a protein having an amino acid sequence of the formula. The DNA can be obtained by a solution or solid synthesis.



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CLAIMS

[Claim(s)]
[Claim 1] The isolated DNA compound including the DNA array which is carrying out the code of the Homo sapiens inflow peptide transporter activity.
[Claim 2] The isolated DNA compound including the DNA array which is carrying out the code of the protein which has the amino-acid-residue array of the array number 1 according to claim 1.
[Claim 3] The isolated DNA compound including the DNA array of the array number 2 according to claim 2.
[Claim 4] A recombinant DNA vector including a DNA array according to claim 1, 2, or 3.
[Claim 5] The host cell by which the transformation was carried out by the recombinant DNA vector according to claim 4.
[Claim 6] The DNA array which is carrying out the code of the Homo-sapiens inflow peptide transporter activity which installed so that it might be discovered from promotor and translation activation array; which performs the transformation of a host cell using the recombinant DNA expression vector containing (a) below approach: (1) which consists of a process of the following which makes Homo-sapiens inflow peptide transporter activity discover, and (b), and functions in a :(a) this host cell, (b) this promotor, and a translation activation array;
(2) Cultivate this host cell by which the transformation was carried out in the process (1) under the suitable conditions for the manifestation of Homo sapiens inflow peptide transporter activity.
[Claim 7] The approach according to claim 6 by which the transformation of the host cell is carried out by the recombinant DNA expression vector according to claim 4.
[Claim 8] How to consist of the following processes for measuring intracellular incorporation of a compound: By the recombinant DNA expression vector which brings about the manifestation of (a) Homo sapiens inflow peptide transporter activity, contact the cell and this compound by which the transformation was carried out, and carry out assay about; and transportation of this compound to the inside of (b) this cell.
[Claim 9] The approach according to claim 8 by which the transformation of the cell is carried out by the recombinant DNA expression vector according to claim 4.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention -- a recombination deoxyribonucleic acid (following "DNA") -- it is related with the field of law. This invention offers the isolated DNA compound containing DNA which is carrying out the code of the inflow peptide transporter (following "inflow peptide transporter") activity of a proton-dependency. Furthermore, a recombinant DNA vector and a host cell are offered.
[0002]
[Description of the Prior Art] In a mammals cell, a peptide is conveyed out of intracellular and a cell by the transporter from which some differ. The transporter which bears functionally an outflow out of the transporter which bears the intracellular inflow of a peptide, and the cell of a peptide exists. An inflow transporter conveys a small peptide and the compound of relation into cytoplasm, and they are carrying out indirect linking to the energy source through ion inclination. An outflow transporter consists of a transporter from which some which function as removing a peptide from cytoplasm differ. [Endicott and Ling by which P-glycoprotein which removes many oncolysis objects and hydrophobic peptides is contained in these, 1989, Annu.Rev.Biochem.58:137-171; Sharma and others, 1992, J.Biol.Chem.267 : 5731-5734].
[0003] This invention relates to the peptide transporter which bears the inflow of the peptide to a cell or organelle. The peptide transporter of this class A gastrointestinal tract, the kidney, [Ganapathy and others located in a placenta and liver lysosome, 1991, and Indian J.Biochem.Biophys.28 Am.J.Physiol. : 317-323; -- Skopicki and others and 1991 -- 261 : F670-F678; Ganaopathy and others, 1981, J.Biol.Chem.256 : 118-124; Bird and Lloyd, 1990, Biochim.Biophys.Acta 1024 : 267-270].

[0004] Usually, an inflow peptide transporter is located in the brush border of the epithelial cell of membrane. The property of a transporter is studied among an intestinal-mucosa preparation object in the location of a basis, and is further studied by in vitro one using the brush-border-membrane vesicle, the isolated intestines cell, and the cell culture object. A rat, a hamster, a rabbit, a fowl, The preparation object obtained from a Japanese newt and Homo sapiens [Ganapathy and Leibach to which it uses for and research is done, 1991, Curr.Biol.3 : 695-701; Said and others, 1988, Biochim.Biophys.Acta 941 : 232-240; Kramer and others and 1988, Biochim.Biophys.Acta 939 : 167-172; Colonge and others, 1990 and Am.J.Physiol. 259 : G775-G780; Shimada and Hoshi, 1986, Jpn.J.Physiol.36 : 451-465; Matthews and Burston, 1984, and Clinical Sci., 67 : 541-549]. A small peptide (JI and tripeptide), an antibiotic (some oral beta-lactams are included), Oral angiotensin converting enzyme (ACE) inhibitor, The solute from which many containing oral renin inhibitor differ and by the inflow peptide transporter [Ganapathy and Leibach which are conveyed into the cytoplasm of an intestines cell, 1991, and Curr.Biol.3 : -- 695-701; Okano and others, 1986, and J.Biol.Chem.261 : 14130-14134; Nakashima and others, 1984, Biochem.Pharm.33:3345-3352; Muranushi and others, 1989, Pharm.Res.6 : 308-312; Friedman and Amidon, 1989 and Pharm.Res.6 : 1043-1047; Friedman and Amidon, 1990, J.Control.Rel.13 : 141-146; Kramer, 1991, and 17 th International Congress of Chemotherapy, June 23-28, Berlin, F.R.G., Abstract No.1415].
[0005] An inflow peptide transporter plays a central role in absorption of the oral medicine object

containing beta-lactam and ACE inhibitor of a certain kind. The inflow peptide transporter was able to distinguish what is absorbed in taking orally in Homo sapiens, and a thing without that right among 27 sorts of investigated beta-lactam antibiotics [Tabas and others, 1991, and 31 st Interscience Conference on Antimicrobial Agents and Chemotherapy Abstract No.164]. Furthermore, the thing for which a parenteral beta-lactam antibiotic is not conveyed although an inflow peptide transporter conveys many oral beta-lactam antibiotics In the research using Homo sapiens intestines Caco-2 cell and the rabbit intestines brush film [Dantzig and others shown and 1992, Biochim.Biophys.Acta 1112 : 167-173; Dantzig and others, 1992 and 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, CA, and Abstract No.1460; Snyder and others and 1992, 32 nd Interscience Conference on Antimicrobial Agents and Chemotherapy Abstract No.1461; Okano and others, 1986, J.Biol.Chem.261:14130-14134]. Same research which predicts oral absorption of which ACE inhibitor the capacity of an inflow peptide transporter is investigated and is carried out is done [Friedman and Amidon, 1989, and Pharm.Res.6:1043-1047].

[0006] Inflow peptide transporters are a sodium dependency and energy dependence. Symport of the proton is carried out with a substrate ("proton-dependency"). A substrate [Hoshi which presents the capacity condensed to intracellular to level higher than the level which exists out of a cell, 1986, Ion Gradient-Coupled Transport, and INSERM symposium No.26. Editors: F.Alvarado and C.H.van Os, Elsevier Science Publishers; Ganapathy and Leibach, 1991, and Curr.Opinion Cell Biol.3:695-701; Ganapathy and others, 1991, Indian J.Biochem.Biophys.28 : 317-323]. The substrate specificity of an inflow peptide transporter is investigated in some kinds. Although these are not the same As very similar [Inui and others who comes out and exists, 1992, and J.Pharmacol.Exp.Thera.260 :482-486; Ganapathy and Leibach, 1983, and J.Biol.Chem.258 : 14189-14192; Yasumoto and Sugiyama, 1980 and Agric.Biol.Chem. 44 : 1339-1344; Nakashima and others, 1984, Biochem.Pharmacol.33 : 3345-3352; Okano and others, 1986, Biochem.Pharmacol.35 : 1781-1786]. The binding site of an inflow peptide transporter is not known, therefore its description of the absolute chemical structure required for association and transportation of a solute is also strange. research of correlation of the structure of a substrate and inhibitor and activity is done, and it is unnecessary to transportation -- [as which the structural description of shoes is solved -- Bai and others, 1991, and Pharm.Res.8 : -- 593-599; Snyder and others, 1992, 32 nd Interscience Conferenceon Antimicrobial Agents and Chemotherapy, Oct.11-14, Anaheim, CA, and Abstract No.1461].

[0007] Inflow peptide transporter activity is [Kramer identified as 127,000dalton membrane protein from rabbit intestinal mucosa by the optical affinity-labeling method using the penicillin or the cefalexin analog by which the radiation label was carried out by which the radiation label was carried out, 1987, and Biochim.Biophys.Acta 905:65-74.; Kramer and others, 1988, Biochem.PHarmacol.37 : 247-2435]. [which the 127,000dalton protein of purification of the rabbit intestinal-mucosa preparation object origin reconfigurated in liposome gave association and transportation activity -- Kramer and others, 1990, and Biochim.Biophys.Acta 1030 : -- 50-59]. [by which the rabbit inflow peptide transporter is functionally discovered in Xenopus laevis oocyte -- Miyamoto and others, 1991, and J.Biol.Chem.266 : -- 4742-4745]. However, the structure of the cloning gene which is carrying out the code of the mammalian inflow peptide transporter, or one of its components are not reported to any kinds.

[0008] Probably, cloning of an inflow peptide transporter will be useful for development of the approach of enabling the quick identification and the development of an oral absorption drug which use this device. An oral bioavailability is the very desirable property of many drugs. Probably, especially measurement of the oral bioavailability of the drug in the phase in early stages of development will be advantageous. Current and a drug are first evaluated about an oral bioavailability in the animal model. This process needs selection of a small number of compound very much, and composition of this compound must be expanded to extent evaluated in these models. When a compound is not absorbed in taking orally using these models, in order to attain an oral bioavailability, the analog of the compound is created in many cases. This process wastes time amount, is difficult and requires costs. Furthermore, although you may set to the animal model and it is absorbed, there are many examples of the compound which is not absorbed by Homo sapiens. Since it supplements with this conventional approach, other evaluation approaches are needed.

[0009]

[Problem(s) to be Solved by the Invention] Especially this invention offers the host cell by which the transformation was carried out by the isolated DNA compound including the DNA array which is carrying out the code of the mammalian inflow peptide transporter activity, the recombinant DNA expression vectors which are carrying out the code of the mammalian inflow peptide transporter activity, and these recombinant DNA expression vectors. These recombinant DNA expression vectors and host cells are useful in the approach for making inflow peptide transporter activity discover. (a) below : (1) which this approach becomes from the following processes And (b) So that it may be discovered from promotor and translation activation array; which performs the transformation of a host cell using the included recombinant DNA expression vector, and functions in a :(a) this host cell, (b) this promotor, and a translation activation array DNA array which is carrying out the code of the inflow peptide transporter activity of the installed mammals;
(2) Cultivate this host cell by which the transformation was carried out in the process (1) under the suitable conditions for the manifestation of inflow peptide transporter activity.
[0010] Probably, the capacity which predicts the taking-orally-availability of the drug in Homo sapiens by the initial stage of a drug discovery process will be advantageous. For this purpose, this invention offers a useful analysis means in the prediction of the oral availability of the physic compound in Homo sapiens by the inflow peptide transporter. That is, by the recombinant DNA expression vector which brings about the manifestation of :(a) mammals inflow peptide transporter activity about the approach for measuring incorporation of the compound by the cell which consists of the following processes, one mode of this invention contacts the cell and this compound by which the transformation was carried out, and carries out assay about; and transportation of this compound to the inside of (b) this cell.
[0011] Other modes as these modes of this invention and a hybridization probe of the DNA array of this invention, such as use, are explained in more detail below, and are indicated to a claim.
[0012]
[Means for Solving the Problem]
Definition coding array: The DNA array in the reading frame of the gene which carries out the code of the amino-acid-residue array of the protein discovered from a gene.
DHFR: Dihydrofolate reductase gene.
Gene: The DNA segment containing the promotor who installed so that a gene product might be made to discover, a translation activation array, a coding array, and 3' regulatory sequence.
Inflow peptide transporter activity: Migration of the substrate which crosses the film depending on existence of the proton gradient of internal directivity. Functionally, activity can be measured by measuring transportation of the compound which crosses the film under the absence of the excessive amount of the known substrate (for example, [a small peptide (for example, JI and tripeptide), an antibiotic (for example, cefalexin), oral angiotensin converting enzyme (ACE) inhibitor, and oral renin inhibitor]) of a transporter, or existence under existence of pH-inclination (that is, external one has acidity higher than the interior of a cell or a membrane vesicle) of internal directivity.
Promotor: The DNA array which makes the imprint of DNA order or start.
Recombinant-DNA expression vector: It is all the DNA matter that reproduces autonomously or performs a nest, although a plasmid is included, it is not limited to it, but the promotor and other regulatory sequences which were installed so that the DNA segment which carries out the code of a polypeptide or the RNA might be made to discover are included.
Recombinant-DNA array: It is all the DNA arrays that excepted the host chromosome which drew DNA, and include the DNA array by which isolation and composition or partial composition was carried out.
restriction fragment: -- all the lines produced according to an operation of the restriction enzyme beyond 1 or it -- a DNA molecule.
Translation activation array: The accommodation DNA array which promotes a translation in the protein of mRNA when it imprints to mRNA.
all the nucleotides and amino acid abbreviation which were used by this detail letter -- an United States patent trademark station -- 37 C.F.R.Section -- as shown in 1.822 (b) and (1992), it accepts.
[0013] The restriction enzyme and functional map which were shown in the explanatory view side of a drawing are the near display of the recombinant DNA vector indicated in this specification. The

information on a limit part is not comprehensive. The restriction enzyme part of many predetermined molds may exist from the actually shown part on a map. Drawing 1 is the restriction enzyme part and functional map of a plasmid pPSJ179. Drawing 2 is the restriction enzyme part and functional map of a plasmid pPSJ189.

[0014] Detailed explanation this invention offers the isolated DNA compound including the DNA array which is carrying out the code of the mammals inflow peptide transporter activity. The amino acid sequence of an inflow peptide transporter is shown as an array number 1 among an array table. The DNA array which is carrying out the code of the inflow peptide transporter is shown as an array number 2 among an array table.

[0015] This contractor will admit that it is possible to build the DNA array from which many which carry out the code of the array number 1 differ with the property of the degeneracy of a gene code. The DNA array shown by the array number 2 is one [mere] of many possible inflow peptide transporter coding arrays. therefore, the structure indicated in the following and the attached example about the desirable DNA compound, vector, and transformant of this invention -- the thing for instantiation -- it is -- it does not pass and does not have the intention of limiting the range of this invention.

[0016] An array can be prepared by various approaches, therefore is limited to no specific preparation means now when the array of an inflow peptide transporter became known. the DNA array of this invention -- a DNA synthesis method, cDNA cloning, genome cloning, and polymerase chain reaction (PCR) -- it can manufacture by law or some approaches including the combination of these approaches. these and other approaches -- Maniatis[-- "molecular cloning : -- -- a laboratory -- a manual -- " -- Cold Spring Harbor Press -- ColdSpring Harbor Laboratory -- Cold Spring Harbor -- New York --] (1989) -- or -- F . -- M . -- Ausbel -- ** -- [-- "-- molecular biology -- recently -- a protocol -- " -- 1989 --] -- indicating -- having -- **** . The contents of both bibliography of these shall constitute some of these specifications.

[0017] The DNA array of this invention is compoundable using an approach and equipment available as a commercial item. For example, the DNA array of this invention can be manufactured using a solid phase phosphotriester method. A DNA array is compoundable with the amelioration phosphotriester method using the DNA construction block protected completely. such a synthesis method -- substantial -- Itakura et al. -- [1977 and Science 198 : 1056] and Crea[-- Proc.Natl.Acad.Sci.U.S.A.75 : 575], and Narang et al. -- [1980 and Methods in Enzymology 68 : It can carry out according to the approach of 90]. It adds to the approach by handicraft and is ABS 380A. A DNA array is compoundable using automation synthesizer units, such as a DNA synthesizer (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404). Moreover, polymerase chain reaction can be made to generate a DNA array. For example, refer to the United States patent numbers 4,800,159 and 4,683,202 and the Europe patent public presentation number 0258017 (March 2, 1987 public presentation).

[0018] It can be known widely, and can get down and the approach for a solution and solid phase composition can use various automation synthesizer units available as a commercial item according to a known protocol. for example, Stewart and Young [Solid Phase Synthesis 2nd edition, Pierce Chemical Company, and 1984]; Tam et al. -- [1983 and J.Am.Chem.Assoc.105 : 6442];, and Merrifield et al. -- [1982 and Biochemistry 21 : 5020] -- reference.

[0019] DNA which is carrying out the code of the mammalian inflow peptide transporter activity can be cloned in various vectors by the well-known approach for the time being in the fields. Many suitable vectors including cosmid, a plasmid, a bacteriophage, a baculovirus, and a virus can be used. One of the main requirements to such a vector is being able to carry out the transformation of self's being reproduced and the host cell. Preferably, this vector is a recombinant DNA expression vector which may make the inflow peptide transporter activity of the mammals in which a code is carried out by the DNA array of this invention discover. The usual expression vector contains at least promoterregion, a 5 '- untranslation region, coding array, and 3'-untranslation region, a replication origin, a selective marker, and the conclusion section of an imprint. Furthermore, although the array in which a useful vector presupposes that it is possible the reproduction in Escherichia coli is included by this invention, it is usually because it is much more efficient that this prepares plasmid DNA in E.coli from other host organisms.

[0020] The various expression vectors which can be embellished in order to make the new DNA array of this invention discover exist. It does not have the intention of only mentioning as an example the specific vector illustrated in this specification, and limiting the range of this invention. the discovered method -- Maniatis[-- "molecular cloning It is indicated by :laboratory manual"] or the "latest protocol of molecular biology" [16.3-17.44] (1989). Moreover, the discovered method in Saccharomyces is indicated by "the latest protocol of molecular biology" (1989).
[0021] Prokaryon nature vectors, such as a pNH vector (StratageneInc., 11099 N.Torrey Pines Rd., LaJolla, CA 92037), a pET vector (Novogen Inc., 565 Science Dr., and Madison WI 53711), and a pGEX vector (Pharmacia LKB Biotechnology Inc., Piscataway, and NJ 08854), are contained in the vector suitable for using it in the case of this invention operation. For the example of an eukaryon nature vector useful in the case of this invention operation Vector pRc/CMV, pRc/RSV and pREP (it Invitrogen(s)) 11588 Sorrento Valley Baculovirus vectors, such as Rd., San Diego, CA 92121;pVL1392, and pVL1393, pAC360 (Invitrogen); YRP17, YIP5, and YEP24 () [New] Yeast vectors, such as England Biolabs, Beverly, and MA, And Picchia vectors, such as pRS403, pRS413 (Stratagene Inc.), and pHIL-D1 (Phillips Petroleum Co., Bartlesville,-74004), are contained.
[0022] A functional promotor is contained in the promotor for using it in the expression vector of this invention in a prokaryotic cell or an eukaryotic cell. In a prokaryotic cell, a lactose (lac) control member, a bacteriophage lambda (pL) control member, an arabinose control member, a tryptophan (trp) control member, bacteriophage T7 control members, and these hybrids are contained in a functional promotor. In an eukaryotic cell, Saccharomyces promotors, such as Picchia promotors, such as baculovirus promotors, such as an Epstein Barr virus promotor, an adenovirus promotor, an SV40 promotor, a Rous-sarcoma-virus promotor, a cytomegalovirus, and an AcMNPV polyhedron promotor, and an alcohol oxidase promotor, a gal4 inductivity promotor, and a PGK compositionality promotor, are contained in a functional promotor.
[0023] Furthermore, the vector of this invention can contain one of the various markers of a large number which make easy selection of a host cell by which the transformation was carried out of arbitration. The gene related to the enzyme relevant to temperature sensitivity, drug tolerance, or the phenotype property of a host organism is contained in such a marker.
[0024] After inserting into a vector the DNA array which is carrying out the code of the mammalian inflow peptide transporter activity, the vector can be used and the transformation of the host cell can be carried out. Usually, the cellularity living thing containing the prokaryotic cell or eukaryotic cell by which a transformation may be carried out to a host cell by the vector containing DNA of this invention is included. The transformation of a cell and the approach of transfection are common knowledge for the time being in the fields, and can be found out in common bibliographies, such as Maniatis et al. (1989) and the "latest protocol of molecular biology" (1989).
[0025] This invention is not limited to the use to a specific host cell. The vector of this invention can be introduced into many host cells, and can be made to discover. The host cell to which the transformation of this invention was carried out can be cultivated in the usual nutrition culture medium embellished suitably because of induction of a promotor, selection of a transformant, or magnification of a gene. Probably culture conditions, such as temperature and pH, are conditions already used about the host cell chosen for the manifestation, and will be clear to this contractor.
[0026] It depends for selection of a specific host cell on the specific expression vector used in order to make the inflow peptide transporter activity-coding DNA compound of this invention discover to some extent. After introducing to the host cell of the vector of this invention, a transformant can be chosen based on selectable phenotype. The selectable marker which exists on an expression vector can give this selectable phenotype.
[0027] In a suitable host cell, for example, prokaryotic cell; Chinese hamster ovary cell CHO-DHFR-[American Type Culture Collection, such as Escherichia coli and Bacillus subtilis (ATCC), From 12301 Parklawn Drive, Rockville, and Maryland 20852-1776, under trust number ATCC CRL-9096 Available], Chinese hamster ovary cell CHO-K1 (ATCC CCL-61), The Syrian hamster cell AV12 (ATCC CRL 1573), A Homo sapiens lymphocyte CCRF-CEM cell, a Homo sapiens neuroblastoma cell, the Buta kidney cell (LLC-PK1, ATCC CL101), and liver, A brain, Eukaryotic cells, such as the skin and a cell of the suprarenal gland origin; yeast cell;Spodoptera frugiperda Sf9 () which reaches Saccharomyces cerevisiae and contains Picchia pastoris [ATCC] The insect cell

containing the Leucania larva cell of CRL 1711 etc.; a fungus cell including an Aspergillus kind is contained.

[0028] the manifestation in a prokaryotic cell and an eukaryotic cell -- Maniatis et al. (1989), Kaufmann["the principle of gene engineering, and an approach", and J.K -- the volume on Setlow, and Plenum Press 9 : It is indicated by 155 and] (1988). The manifestation of yeast is indicated by Barr and others [the volume gene engineering" of "yeast, and on Butterworth, and Boston 1989]. The manifestation in an insect cell is Maeda[1989, "the annual report of entomology", and 34. : It is indicated by 351].

[0029] The DNA array shown by the array number 2 was acquired from the cDNA clone prepared from mRNA of Caco-2 cellular in. Caco-2 cellular in is [Dantzig and Bergin which are the Homo sapiens colonic gland cancer cellular in where incorporating an antibiotic by the inflow peptide transporter is shown, 1990, and Biochim.Biophys.Acta 1027. : 211-217; Dantzig and others, 1992, Biochim.Biophys.Acta 1112 : 167-173]. Caco-2 cell is available under ATCC to the trust number ATCC HTB37.

[0030] The instantiation vector of this invention was introduced into Escherichia coli RR1 or E.coli DH5 alpha cell, and it ****ed to Northern Regional Research Laboratories (NRRL) (Peoria and Illinois 61604) on January 21, 1993, and carried out to a part of permanent storage culture collection. A specific culture and a specific trust number are shown in Table 1.

[Table 1]

Table [ ] 1 culture Trust number E.coli K12 -- DH5 alpha/pPSJ 179 NRRL B-21041 E.coli K12 RR1/pPSJ189 NRRL B- 21042 [0031] A culture comes to hand and a plasmid is isolated with a conventional method. Subsequently, in order to make a mammals inflow peptide transporter produce, this plasmid can be introduced into a direct host cell.

[0032] Die length is about 8500 base pairs, and a plasmid pPSJ179 contains DNA which is carrying out the code of the inflow peptide transporter of the Caco-2 cell origin. The plasmid pPSJ179 built the 3.4 kb XbaI-HindIII cDNA restriction enzyme fragmentation containing inflow peptide transporter-coding DNA by carrying out cloning into vector pRc/RSV (Invitrogen) available as a commercial item. Since the inflow peptide transporter had the HindIII restriction enzyme part inside, it used partial restriction enzyme digestion in cloning of 3.4kb XbaI-HindIII fragmentation. A plasmid pPSJ179 contains the inflow peptide transporter gene installed so that it might be discovered from the neomycin resistance gene and Rous-sarcoma-virus (RSV) promotor for the selection in the ampicillin resistance gene for the selection in Escherichia coli, and an eukaryotic cell. The restriction enzyme and functional map of a plasmid pPSJ179 are shown in attached drawing 1 .

[0033] A plasmid pPSJ189 is also the example of the vector of this invention. A plasmid pPSJ89 is the magnitude of about 12.2 kilobases. A plasmid pPSJ189 contains the KpnI-SpeI restriction fragment of the 3.4 kilobase pairs containing inflow peptide transporter-coding DNA cloned in the variant with which Plasmid pHD was embellished. Plasmid pHD was embellished so that the restriction enzyme part for making easy cloning of the KpnI-SpeI restriction fragment of the 3.4 kilobase pairs containing inflow peptide transporter coding DNA might be included. Plasmid pHD is indicated in the Europe patent public presentation number 0245949 (November 19, 1987 public presentation). A plasmid pPSJ189 contains BK enhancer and the adenovirus main late promoters which were installed for the hygromycin tolerance gene for the selection in the ampicillin resistance gene for the selection in Escherichia coli, and an eukaryotic cell, a DHFR gene, and inflow peptide transporter gene expression. The restriction enzyme and functional map of a plasmid pPSJ189 are shown in attached drawing 2 .

[0034] This contractor will admit that inflow peptide transporter coding DNA can be started as various restriction enzyme fragmentation from plasmids pPSJ179 and pPSJ189, and may be cloned in many expression vectors. For example, the inflow peptide transporter coding activity DNA can be started as KpnI-SpeI restriction enzyme fragmentation of 3.4 kilobase pairs from a plasmid pPSJ189 as 3.4 kilobase-pair HindIII-XbaI restriction enzyme fragmentation from a plasmid pPSJ179. This contractor will accept the thing for which partial restriction enzyme digestion will be required, in order to prepare the DNA fragment which carries out the code of the perfect inflow peptide transporter to because of that of the existence of many restriction enzyme parts in plasmid DNA. Identification, isolation, and the approach for carrying out cloning are common knowledge for the time being in the

fields about various restriction enzyme fragmentation including the inflow peptide transporter coding activity DNA.

[0035] other transporters by which structure is similar to the inflow peptide transporter of this invention based on the indication of this specification -- polymerase chain reaction (PCR) -- it can identify with the combination of the approaches of common knowledge, such as law and DNA hybridization, or these approaches. An inflow peptide transporter consists of an extracellular field (about amino acid residue 1-778 of the array number 1), and a transformer MENN bulan field (about amino acid residue 778-809 of the array number 1). An extracellular field is [Takeichi and M. which are very much related to the family of the protein known as cadherin, 1990, and Annu.Rev.Biochem.59. : 237-252]. A cadherin family has the outside of a highly preservable cell, and an intracellular field. However, an inflow peptide transporter is [Klinter which does not have the intracellular field of shelf life where it is shown that it is required for the functional activity of cadherin, 1992, and Cell 69. : 225-236]. The protein related to an inflow peptide transporter can be identified using the hybridization method based on this difference between cadherin and an inflow peptide transporter.

[0036] Under a certain hybridization method, a probe specific to a cadherin family and the shelf-life extracellular field of an inflow peptide transporter, and the shelf-life intracellular field of b cadherin family is obtained. Such a probe can be obtained using the PCR method. In this case, template DNA may be cDNA obtained from the cellular in of a different tissue form which discovers genomic DNA or inflow peptide transporter activity, and cadherin. The kidney, an intestinal tract, the cell of the pancreas origin, or the endothelial cell of the "blood-brain" gateway origin is contained in the possible source of supply for template DNA.

[0037] A specific probe is first used for the highly preservable extracellular field of a cadherin family and an inflow peptide transporter in a hybridization experiment, and the gene which has the extracellular field of cadherin and other peptide transporters is identified. Subsequently, the gene which carries out the code of the cadherin is identified, using the probe obtained from the intracellular field of cadherin as a hybridization probe. Although it reacts with the probe to an extracellular field, the candidate of an inflow peptide transporter deserves the gene to which the probe to an intracellular field does not react. These genes are cloned in a recombinant DNA expression vector, and it introduces into a suitable host cell. Subsequently, assay of the host cell by which the transformation was carried out is carried out about the manifestation of inflow peptide transporter activity.

[0038] The same result can be mentioned using a different-species hybridization method. In this case, a possible inflow peptide transporter is distinguished from cadherin using the DNA fragment showing a part for the outside of the cell of cadherin, and the interior of a cell.

[0039] Other genes which are carrying out the code of the peptide transportation activity using the conventional hybridization method using DNA which is carrying out the code of the inflow peptide transporter of this invention, or the probe based on the part of the arbitration can be identified. For example, the gene which has peptide transportation activity using the probe based on the array number 2 or its part can be identified. Moreover, the gene which has peptide transportation activity using the probe based on the amino acid sequence of the array number 1 or its part which degenerated can be identified. Maniatis and others (1989) is indicating the hybridization method.

[0040] As shown above, this invention offers the approach for measuring incorporation of the compound by the inflow peptide transporter. This approach is useful in prediction of the oral bioavailability of the compound by the inflow peptide transporter in Homo sapiens. A variety of compounds can be examined about the incorporation by the inflow peptide transporter. A small peptide and a small remedy object, for example, an antibiotic, ACE inhibitor, and renin inhibitor are contained in the example of such a compound. These compounds are mere instantiation. This approach is applicable to any compounds as a matter of fact, in order to examine the capacity incorporated by the inflow peptide transporter. Therefore, in one mode, this invention contacts the cell and this compound by which the transformation was carried out by the recombinant DNA expression vector which brings about the manifestation of :a mammals inflow peptide transporter activity which offers the approach for measuring incorporation of the compound to the inside of a cell of consisting of the following processes, and carries out assay about transportation of this compound

to the inside of b this cell.
[0041] The example of the recombinant DNA expression vector which brings about the manifestation of useful inflow peptide transporter activity in the approach of this invention was indicated above. Such a recombinant DNA expression vector can be adjusted for the optimal manifestation of the inflow peptide transporter activity in the host cell chosen for a manifestation.
[0042] The various cells containing the cell indicated above can be used in this approach. Especially the cell that lacks inflow peptide transporter activity before the transformation in the recombinant DNA expression vector of this invention is useful in this method. The cell which has incorporation of a measurable compound before the transformation in the recombinant DNA expression vector of this invention is also useful. Assay of the cell by which the transformation was carried out by the recombinant DNA expression vector which is carrying out the code of the inflow peptide transporter activity in which case can be carried out about increase of transportation of the trial compound to this cell.
[0043] In this mode of this invention into a useful cell For example, prokaryotic cells, such as Escherichia coli and Bacillus subtilis; Chinese hamster ovary cell CHO-DHFR-[American Type Culture Collection (ATCC), From 12301 Parklawn Drive, Rockville, and Maryland 20852-1776, under trust number ATCC CRL-9096 Available], Chinese hamster ovary cell CHO-K1 (ATCC CCL-61), The Syrian hamster cell AV12 (ATCC CRL 1573), A Homo sapiens lymphocyte CCRF-CEM cell, a Homo sapiens neuroblastoma cell, the Buta kidney cell (LLC-PK1, ATCC CL101), and liver, A brain, Eukaryotic cells, such as the skin and a cell of the suprarenal gland origin; yeast cell;Spodoptera frugiperda Sf9 () which reaches Saccharomyces cerevisiae and contains Picchia pastoris [ATCC] The insect cell containing the Leucania larva cell of CRL 1711 etc.; a fungus cell including an Aspergillus kind is contained. Moreover, probably, the peptide transportation deletion mutant of the cell which made reference upwards will be useful in the approach of this invention. such a peptide transportation deletion mutant -- Escherichia coli[-- DeFelice and others, 1973, and J.Bacteriol.116 : 751-7560] and yeast [-- Island and others, 1991, and Curr.Genet.20 : 457-463; Marder and others, 1978, and J.Bacteriol.136 : It is indicated about 1174-1177].
[0044] Probably, the specific vectors used in order to make an inflow peptide transporter discover as shown above differ according to the host cell to be used.
[0045] Incorporation of the compound by the transformer FEKUTANTO cell which has discovered inflow peptide transporter activity can be measured by various approaches. measurement of an appearance of a trial compound host intracellular to these approaches, i.e., this cell, -- dissolving -- a melt sample -- a high speed liquid chromatography -- or when the radiation label of this compound is carried out, measurement by analyzing a compound by detection of radioactivity is included. Moreover, other properties related to a specific trial compound can be measured. That is, in order to screen a specific compound, the assay usually used can be used. for example, the capacity of a compound to permute association of the ligand to a receptor in receptor assay (or enhancement), the capacity of the compound which checks a related enzyme (or stimulus), the capacity of the compound which checks growth of a living thing (or stimulus), or a trial compound has -- will come out and I will be -- being of a certain kind -- others -- a property can be used. Inflow peptide transporter activity can be measured using various assays containing the assay currently indicated by Bradner and Claridge [volume 1984, "screening system in antineoplastic drug", and on W.A.Remers, Wiley-Interscience Pub., John Wiley and Sons, and Inc.N.Y., and NY].
[0046]
[Example] It has the intention of the example shown below helping much more understanding of this invention. The matter, the specific kind, and the specific conditions of being used have the intention of explaining this invention in more detail, and do not tend to limit the just range of this invention. The approach for actuation of DNA and analysis was performed as essentially indicated by Maniatis and others (1989). The conditions for a restriction enzyme reaction are manufacturer [Boehringer Mannheim (BM), Indianapolis, and IN.; New England Biolabs (NEB), Beverly, MA; The conditions currently recommended by Bethesda Research Labs (BRL), Gaithersburg, and MD] were used.
[0047] Transfection of the example 1 Chinese hamster ovary cell (CHO-K1 and ATCC CCL 61) was carried out by the plasmid pPSJ179 using the calcium precipitate protocol indicated in the Stratagene mammalian transfection kit (Stratagene Catalog # 200285). A plasmid pPSJ179 can be

isolated from Escherichia coli K12 DH5 alpha/pPSJ 179 (NRRL B-21041) using the usual alkali-SDS method (Maniatis and others, 1989). The transfection method of a calcium precipitate protocol was performed as follows. It is calcium of 20microg mostly about CHO-K1 cell (one day after 100mm culture plate and plaiting) of complete growth. - It incubated for 20 minutes at 37 degrees C with the DNA sample which precipitated. The DNA sample was either a plasmid pPSJ179 or plasmid pRc/RSV as contrast. Then, this cell was proliferated for three days in F12 culture medium which contains fetal calf serum (Hyclone Laboratories Inc., Logan, UT 84321) 10%. The culture medium was permuted by the growth medium which contains a selection drug and G-418 sulfate (Gibco, Grand Island, NY) by ml in 300microg /, and the cell was proliferated for 13 days at 37 degrees C among 3% CO2 incubator next. The colony chosen for next research was proliferated in the selected time amount and the 37-degree C selected selective medium among 5% CO2 incubator. Transformer FEKUTANTO was evaluated about the manifestation of an inflow peptide transporter using the reactant monoclonal antibody to enzyme joint immune absorbance assay (ELISA) and an inflow peptide transporter. It chose for transportation research of the clone which discovered the inflow peptide transporter antigen of level higher than contrast. according to an exception method -- Dantzig et al. -- [1990 and Biochim.Biophys.Acta 1027 : Choose a clone about the manifestation of an inflow peptide transporter using the approach indicated by 211-217]. Furthermore, the clone containing DNA which is carrying out the code of the inflow peptide transporter can be identified using the hybridization method using the probe based on the array number 1.

[0048] The clone chosen in the example 2 example 1 was evaluated about incorporation of antibiotic cefalexin. Cefalexin is available from Eli Lilly and Company (Indianapolis, IN). CHO-K1 cell (per well - 0.5 to 1x105 cells) by which transfection was carried out -- above -- Costar24- a well -- it was made to increase for three days in a plate The R balanced salt solution (Gibco, Grand Island, NY) (Trans-EBSS) including 25mM HEPES and pH7.4 washed the complete growth cell, it incubated for 45 minutes at 37 degrees C, and, subsequently suction removed Trans-EBSS. This cell was incubated under existence of 1mM [14C] cefalexin among the R balanced salt solution (sodium non-**, Trans-EBSS) of sodium non-** including the 120mM choline chloride, 25mM MES, and pH6.0. Then, washed the cell by ice-cooling Trans-EBSS and pH7.4, it was made to dissolve in 0.2N NaOH, and the part was extracted for scintillation count measurement.

[0049] Typical transformer FEKUTANTO (clone 9) showed incorporation of high [14C] cefalexin more nearly intentionally than contrast. It was shown by next research that incorporation of the cefalexin of 1mM by this transformer FEKUTANTO is checked by the existence of 50mM(s) of Gly-L-Pro (GP) which is the dipeptide which competes with the incorporation by the inflow peptide transporter. By incubating a cell with 1mM [14C] cefalexin and GP, incorporation of the drug in typical transformer FEKUTANTO (clone 9) decreased to the level of a reference cell. Furthermore, transportation of 1mM [14C] cefalexin by the reference cell was not checked by the Gly-L-Pro dipeptide. The result of these researches is shown in Table 2.

[Table 2]

table 2 [ ] A sample Incorporation of 14C-cefalexin (nmol/mg all cell protein) A clone 9 6.6**0.3 Clone 9+GP 4.5**0.03 Contrast 3.3**0.6 Contrast +GP 4.7**0.4 [0050]

[Layout Table]

[0051] array number: -- die-length [of one array]: -- mold [of 832 arrays]: -- amino acid topology: -- class [of straight chain-like array]: -- protein array: -- Met Ile Leu Gln Ala His Leu His Ser Leu Cys Leu Leu Met Leu 1 5 10 15 Tyr Leu Ala Thr Gly Tyr Gly Gln Glu Gly Lys Phe Ser Gly Pro 20 25 30 Leu Lys Pro Met Thr Phe Ser Ile Tyr Glu Gly Gln Glu Pro Ser 35 40 45 Gln Ile Ile Phe Gln Phe Lys Ala Asn Pro Pro Ala Val Thr Phe 50 55 60 Glu Leu Thr GlyGlu Thr Asp Asn Ile Phe Val Ile Glu Arg Glu 65 70 75 Gly Leu Leu TyrTyr Asn Arg Ala Leu Asp Arg Glu Thr Arg Ser 80 85 90 Thr His Asn LeuGln Val Ala Ala Leu Asp Ala Asn Gly Ile Ile 95 100105 Val Glu Gly Pro Val Pro Ile Thr Ile Glu ValLys Asp Ile Asn 110 115 120 Asp Asn Arg Pro Thr Phe Leu Gln Ser Lys Tyr Glu Gly Ser Val 125 130 135 Arg Gln Asn Ser Arg Pro Gly Lys Pro Phe Leu Tyr Val Asn Ala 140 145 150 Thr Asp Leu Asp Asp Pro Ala Thr Pro Asn Gly Gln Leu Tyr Tyr 155 160 165 Gln Ile Val Ile Gln Leu Pro Met Ile Asn Asn Val Met Tyr Phe 170 175 180 Gln Ile Asn Asn Lys Thr Gly Ala Ile Ser Leu Thr Arg Glu Gly 185 190 195 Ser Gln GluLeu Asn Pro Ala Lys Asn Pro Ser Tyr Asn Leu Val 200 205 210 Ile SerVal Lys Asp Met Gly Gly Gln Ser Glu Asn Ser Phe Ser 215 220 225 Asp Thr Thr Ser Val Asp Ile Ile Val

Thr Glu Asn Ile Trp Lys 230 235 240 Ala Pro Lys Pro Val Glu Met Val Glu Asn Ser Thr Asp Pro His 245 250 255 Pro Ile Lys Ile Thr Gln Val Arg Trp Asn Asp Pro Gly Ala Gln 260 265 270 Tyr Ser Leu Val Asp Lys Glu Lys LeuPro Arg Phe Pro Phe Ser 275 280 285 Ile Asp GlnGlu Gly Asp Ile-Tyr-Val-Thr-Gln Pro Leu Asp Arg 290 295 300Glu Glu Lys Asp Ala-Tyr-Val-Phe-Tyr Ala Val Ala Lys Asp-Glu 305 310 315Tyr Gly Lys Pro Leu-Ser-Tyr-Pro-LeuGlu Ile His Val Lys Val 320 325330 Lys Asp Ile Asn Asp Asn Pro Pro Thr Cys Pro Ser Pro Val Thr 335 340 345 Val Phe Glu Val Gln Glu Asn Glu Arg Leu Gly Asn Ser Ile Gly 350 355 360 Thr Leu Thr Ala His Asp Arg Asp Glu Glu Asn Thr Ala Asn Ser 365370 375 Phe Leu Asn Tyr Arg Ile Val Glu Gln Thr Pro Lys Leu Pro Met 380 385 390 Asp Gly Leu Phe Leu Ile Gln ThrTyr Ala Gly Met Leu Gln Leu 395 400 405 AlaLys Gln Ser Leu Lys Lys Gln Asp Thr Pro Gln Tyr Asn Leu 410 415 420 Thr Ile Glu Val Ser Asp Lys Asp Phe Lys Thr Leu Cys Phe Val 425 430 435 Gln Ile Asn ValIleAsp Ile Asn Asp Gln Ile Pro Ile Phe Glu 440 445 450 Lys Ser Asp Tyr Gly Asn Leu Thr Leu Ala Glu Asp Thr Asn Ile 455 460 465 Gly Ser Thr Ile LeuThr Ile Gln Ala Thr Asp Ala Asp Glu Pro 470 475 480 Phe Thr Gly Ser SerLys Ile Leu Tyr His Ile Ile Lys Gly Asp 485 490 495 Ser Glu Gly Arg LeuGly Val Asp Thr Asp Pro His Thr Asn Thr 500 505 510 Gly Tyr Val Ile IleLys Lys Pro Leu Asp Phe Glu Thr Ala Ala 515 520 525 Val Ser Asn Ile Val Phe Lys Ala Glu Asn Pro Glu Pro Leu Val 530 535 540 Phe Gly Val Lys TyrAsn Ala Ser Ser Phe Ala Lys Phe Thr Leu 545 550 555 Ile Val Thr Asp Val Asn Glu Ala Pro Gln Phe Ser Gln His Val 560 565 570 Phe Gln Ala Lys Val Ser Glu Asp Val Ala Ile Gly Thr Lys Val 575 580 585 Gly Asn Val Thr AlaLys Asp Pro Glu Gly Leu Asp Ile Ser Tyr 590 595 600 Ser-Leu-Arg-Gly-Asp Thr Arg Gly Trp Leu-Lys-Ile-Asp-His-Val 605 610 615Thr Gly Glu Ile Phe-Ser-Val-Ala-Pro Leu Asp Arg Glu Ala-Gly 620 625 630Ser Pro Tyr A rg Val Gln Val Val Ala Thr Glu Val Gly Gly Ser 635 640645 Ser Leu Ser Ser Val Ser Glu Phe His Leu Ile Leu Met Asp Val 650 655 660 Asn Asp Asn Pro Pro Arg Leu Ala Lys Asp Tyr Thr Gly Leu Phe 665 670675 Phe Cys His Pro Leu Ser Ala Pro Gly Ser Leu Ile Phe Glu Ala 680 685 690 Thr Asp Asp Asp Gln His Leu Phe Arg Gly Pro His Phe Thr Phe 695 700 705 Ser Leu Gly Ser Gly Ser Leu Gln Asn Asp Trp Glu Val Ser Lys 710 715 720 Ile Asn Gly Thr His Ala Arg Leu Ser Thr Arg His Thr Asp Phe 725 730 735 Glu Glu Arg Ala Tyr Val Val Leu IleArg Ile Asn Asp Gly Gly 740 745750 Arg Pro Pro Leu Glu GlyIle Val Ser Leu Pro Val Thr Phe Cys 755 760 765 Ser Cys Val Glu Gly Ser Cys Phe Arg Pro Ala Gly His Gln Thr 770 775 780 Gly Ile Pro Thr Val Gly Met Ala Val Gly Ile Leu Leu Thr Thr 785 790 795 Leu Leu Val Ile Gly Ile Ile Leu Ala Val Val Phe Ile Arg Ile 800 805 810 Lys Lys Asp Lys Gly Lys Asp Asn Val Glu Ser Ala Gln Ala Ser 815 820 825 Glu Val Lys Pro Leu Arg Ser 830 832 [0052] array number: -- die-length [of two arrays]: -- mold [of 2499 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- DNA array: -- ATGATACTTC AGGCCCATCT TCACTCCCTG TGTCTTCTTA TGCTTTATTT 50 GGCAACTGGA TATGGCCAAG AGGGGAAGTT TAGTGGACCC CTGAAACCCA 100 TGACATTTTC TATTTATGAA GGCCAAGAAC CGAGTCAAAT TATATTCCAG 150 TTTAAGGCCA ATCCTCCTGC TGTGACTTTT GAACTAACTG GGGAGACAGA 200 CAACATATTT GTGATAGAAC GGGAGGGACT TCTGTATTAC AACAGAGCCT 250 TGGACAGGGA AACAAGATCT ACTCACAATC TCCAGGTTGC AGCCCTGGAC 300 GCTAATGGAA TTATAGTGGA GGGTCCAGTC CCTATCACCA TAGAAGTGAA350 GGACATCAAC GACAATCGAC CCACGTTTCT CCAGTCAAAG TACGAAGGCT 400CAGTAAGGCA GAACTCTCGC-CCAGGAAAGC-CCTTCTTGTA-TGTCAATGCC 450ACAGACCTGG ATGATCCGGC-CACTCCCAAT-GGCCAGCTTT-ATTACCAGAT 500TGTCATCCAG CTTCCCATGA-TCAACAATGT-CATGTACTTT-CAGATCAACA 550ACAAAACGGG AGCCATCTCT CTTACCCGAG-AGGGATCTCA GGAATTGAAT 600CCTGCTAAGA ATCCTTCCTA TAATCTGGTG ATCTCAGTGA AGGACATGGG650 AGGCCAGAGT GAGAATTCCT TCAGTGATAC CACATCTGTG GATATCATAG 700 TGACAGAGAA TATTTGGAAA GCACCAAAAC CTGTGGAGAT GGTGGAAAAC 750 TCAACTGATC CTCACCCCAT CAAAATCACT CAGGTGCGGT GGAATGATCC 800 CGGTGCACAA TATTCCTTAG TTGACAAAGA GAAGCTGCCA AGATTCCCAT 850 TTTCAATTGA CCAGGAAGGA GATATTTACG TGACTCAGCC CTTGGACCGA 900GAAGAAAAGG ATGCATATGT TTTTTATGCA GTTGCAAAGG ATGAGTACGG950 AAAACCACTT TCATATCCGC TGGAAATTCA TGTAAAAGTT AAAGATATTA 1000 ATGATAATCC ACCTACATGT CCGTCACCAG TAACCGTATT TGAGGTCCAG 1050 GAGAATGAAC GACTGGGTAA CAGTATCGGG ACCCTTACTG CACATGACAG 1100 GGATGAAGAA AATACTGCCA ACAGTTTTCT AAACTACAGG ATTGTGGAGC 1150 AAACTCCCAA ACTTCCCATG GATGGACTCT TCCTAATCCA AACCTATGCT 1200GGAATGTTAC AGTTAGCTAA ACAGTCCTTG AAGAAGCAAG ATACTCCTCA1250 GTACAACTTA ACGATAGAGG

TGTCTGACAA AGATTTCAAG ACCCTTTGTT 1300 TTGTGCAAAT CAACGTTATT GATATCAATG
ATCAGATCCC CATCTTTGAA 1350 AAATCAGATT ATGGAAACCT GACTCTTGCT GAAGACACAA
ACATTGGGTC 1400 CACCATCTTA ACCATCCAGG CCACTGATGC TGATGAGCCA TTTACTGGGA
1450 GTTCTAAAAT TCTGTATCAT ATCATAAAGG GAGACAGTGA GGGACGCCTG
1500GGGGTTGACA CAGATCCCCA TACCAACACC GGATATGTCA TAATTAAAAA1550
GCCTCTTGAT TTTGAAACAG CAGCTGTTTC CAACATTGTG TTCAAAGCAG 1600 AAAATCCTGA
GCCTCTAGTG TTTGGTGTGA AGTACAATGC AAGTTCTTTT 1650 GCCAAGTTCA CGCTTATTGT
GACAGATGTG AATGAAGCAC CTCAATTTTC 1700 CCAACACGTA TTCCAAGCGA AAGTCAGTGA
GGATGTAGCT ATAGGCACTA 1750 AAGTGGGCAA TGTGACTGCC AAGGATCCAG AAGGTCTGGA
CATAAGCTAT 1800TCACTGAGGG GAGACACAAG AGGTTGGCTT AAAATTGACC
ACGTGACTGG1850 TGAGATCTTT AGTGTGGCTC CATTGGACAG AGAAGCCGGA AGTCCATATC
1900 GGGTACAAGT GGTGGCCACA GAAGTAGGGG GGTCTTCCTT AAGCTCTGTG 1950
TCAGAGTTCC ACCTGATCCT TATGGATGTG AATGACAACC CTCCCAGGCT 2000 AGCCAAGGAC
TACACGGGCT TGTTCTTCTG CCATCCCCTC AGTGCACCTG 2050 GAAGTCTCAT TTTCGAGGCT
ACTGATGATG ATCAGCACTT ATTTCGGGGT 2100CCCCATTTTA CATTTTCCCT CGGCAGTGGA
AGCTTACAAA ACGACTGGGA2150 AGTTTCCAAA ATCAATGGTA CTCATGCCCG ACTGTCTACC
AGGCACACAG 2200 ACTTTGAGGA GAGGGCGTAT GTCGTCTTGA TCCGCATCAA TGATGGGGGT
2250CGGCCACCCT TGGAAGG CAT-TGTTTCTTTA-CCAGTTACAT-TCTGCAGTTG
2300TGTGGAAGGA AGTTGTTTCC-GGCCAGCAGG-TCACCAGACT-GGGATACCCA
2350CTGTGGGCAT GGCAGTTGGT-ATACTGCTGA-CCACCCTTCT-GGTGATTGGT
2400ATAATTTTAG CAGTTGTGTT-TATCCGCATA-AAGAAGGATA-AAGGCAAAGA
2450TAATGTTGAA AGTGCTCAAG CATCTGAAGT CAAACCTCTG AGAAGCTGA 2499

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] They are the restriction enzyme part of a plasmid pPSJ179, and the mimetic diagram of a functional map.
[Drawing 2] They are the restriction enzyme part of a plasmid pPSJ189, and the mimetic diagram of a functional map.

[Translation done.]